

Appl. No.: 09/930,905

Filed: 8/16/01

Page 2

Amendments to the Claims:

1. (Previously presented) A method of preparing a chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme, said method comprising:

- a) contacting a stroma free hemoglobin solution with at least one filter, wherein a first filter retains viral particles and allows passage of a filtrate comprising a hemoglobin polypeptide and an endogenous antioxidant enzyme and the filtrate is substantially free of viral contamination;
- b) chemically modifying the filtrate with an agent; and,
- c) isolating the chemically modified hemoglobin solution and the endogenous antioxidant enzyme, wherein at least one of the endogenous antioxidant enzymes retains enzymatic activity.

2. (Previously presented) The method of claim 1, wherein at least one of the endogenous antioxidant enzymes retaining enzymatic activity is selected from the group consisting of a superoxide dismutase, a catalase, and a glutathione peroxidase.

3. (Previously presented) The method of claim 1, wherein said first filter allows the passage of at least 50% of the endogenous antioxidant enzymes present in the stroma free hemoglobin solution.

4. (Previously presented) The method of claim 1, wherein the first filter comprises a 500,000 molecular weight cutoff filter.

5. (Previously presented) The method of claim 1, wherein said first filter reduces the passage of viral particles that are between about 200-25 nm in size.

6. (Previously presented) The method of claim 1, wherein said first filter reduces the passage of viral particles that are 80-100 nm in size.

Appl. No.: 09/930,905

Filed: 8/16/01

Page 3

7. (Previously presented) The method of claim 1, wherein said first filter reduces the passage of viral particles that are between about 80-50 nm in size.

8. (Previously presented) The method of claim 1, where said first filter reduces the passage of viral particles that are between about 50-25 nm in size.

9. (Previously presented) The method of claim 5, wherein said first filter reduces the passage of said viral particles by about 3 to about 10 log units.

10. (Previously presented) The method of claim 1, wherein said first filter produces a filtrate having a viral load reduction of at least 3 log units.

11. (Previously presented) The method of claim 1 further comprising contacting the filtrate with a second filter wherein said second filter allows the passage of the hemoglobin polypeptide and the endogenous antioxidant enzyme and retains virus particles.

12. (Canceled)

13. (Previously presented) The method of claim 1, wherein the agent is a bifunctional modifying agent.

14. (Currently amended) The method of claim 13, wherein said agent is selected from the group consisting of a sebacyl chloride, a glutaraldehyde, a diasprin derivative derivatives, a polyaldehydes polyaldehydes, a polyoxyethylene, a dextran dextrans, and an insulin.

15. (Previously presented) The method of claim 13, wherein the agent is a bifunctional polyoxyethylene.

Appl. No.: 09/930,905

Filed: 8/16/01

Page 4

16. (Previously presented) The method of claim 1, wherein the agent is a mixture of a bifunctional and a monofunctional polyoxyethylene.

17. (Previously presented) The method of claim 15, wherein the chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme is PHP.

18. (Previously presented) The method of claim 1, wherein chemically modifying said filtrate with an agent comprises deoxygenation and pyridoxalation.

19. (Previously presented) The method of claim 1, wherein said chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme comprises a hepatitis A viral titer of less than about 1 TCID₅₀ unit/ml.

20. (Original) The method of claim 1, wherein the chemically modified hemoglobin solution comprises about a 50% to about a 200% increase in endogenous red blood cell antioxidant activity per unit of hemoglobin found in red blood cells.

21. (Previously presented) A method of preparing a chemically modified hemoglobin solution comprising an endogenous antioxidant enzyme, said method consisting of:

- a) contacting a stroma free hemoglobin solution with at least one filter, wherein a first filter retains viral particles and allows passage of a filtrate comprising a hemoglobin polypeptide and the endogenous antioxidant enzyme and the filtrate is substantially free of viral contamination;
- b) chemically modifying the filtrate with an agent; and,
- c) isolating the chemically modified hemoglobin solution and the endogenous antioxidant enzyme.

Claims 22-35. (Cancelled)